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Effect of Antipyrine On the Plant Cell

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THE EFFECT OF ANTIPYRINE ON THE PLANT CELL

by

William Salisbury Ballard

THESIS

For the Degree of Bachelor of Arts

In Botany

In the College of Science

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THIS IS TO CERTIFY THAT THE THESIS PREPARED UNDER MY SUPERVISION BY

William Salisbury Ballard

ENTITLED The Effect of Antipyrine on the Plant Cell

IS APPROVED BY ME AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE DEGREE

OF Bachelor of Arts

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INTRODUCTORY

The work on the animal side done by Galliotti, on the effect of antipyrine on the cell, together with some preliminary work done by Professor Hottes, employing the root of Vicia Faba, was sufficient to indicate the value of this subject for investigation, and the object of this thesis has been to confirm and extend those observations. In this connection, then, I desire to thank Professor Hottes for the assignment of a subject which appears to be of no small worth, and to thank him also for the aid given me in his direction and assistance toward the performance of the work.

METHODS

The beans, after having a little of the integument removed from one edge to facilitate soaking, were placed in water for twenty-four hours, then planted in sawdust and allowed to germinate. When the roots had reached a length of one and one-fourth to one and a half inches they were transferred to a tap water medium where they remained twenty-four hours; the purpose of this being to cause them to become acclimated to a liquid medium. They were then ready for experimentation and the method was simply to transfer the seedlings to a tap-water solution of antipyrine of the desired strength. To this end large glass or granite-ware pans were used. The pans were covered with a screen of one-fourth inch mesh and the seedlings were rapidly taken from the tap-water medium and placed thereon, their roots projecting through into the pan. When all had been transferred, which required only two or three minutes, the solution previously prepared was quickly poured into the dish so that all

were immersed in the antipyrine at practically the same instant. It was desirable to study not only the direct effect of the antipyrine, as would be obtained by killing and fixing the roots immediately after removing from the solution, but also to study the results when a period of recovery was permitted before killing. To this end the method was as follows. After varying intervals, 1/2, 1, 1-1/2, 2, 2-1/4, 2-1/2 hours, two or three seedlings were removed and killed immediately. At these same times others were transferred back to tap-water containing no antipyrine, the roots being first held in a stream of water to wash the antipyrine from their surface. By thus leaving the roots in tap-water they had a chance to recover from the effects of the antipyrine, or "regenerate". This regeneration also, was permitted to go on for different lengths of time for different previous exposures in antipyrine. Thus we may have roots treated in the way indicated in the following table:-

Killed immediately after exposure of,	Killed at end of 5 hrs. regeneration, after previous exposure of,	Killed at end of 12hrs regeneration, after previous exposure of,
1/2 hr.	1/2 hr.	1/2 hr.
1 hr.	1 hr.	1 hr.
1-1/2 hr.	1-1/2 hr.	1-1/2 hr.
2 hrs.	2 hrs.	2 hrs.
2-1/4 hrs.	2-1/4 hrs.	2-1/4 hrs.
2-1/2 hrs.	2-1/2 hrs.	2-1/2 hrs.

Killed at end of 24 hrs. regeneration, after previous exposure of,	Killed at end of 36 hrs. regeneration, after previous exposure of,
1/2 hr.	1/2 hr.
1 hr.	1 hr.
1-1/2 hr.	1-1/2 hr.
2 hrs.	2 hrs.
2-1/4 hrs.	2-1/4 hrs.
2-1/2 hrs.	2-1/2 hrs.

Considerable experimentation was necessary before the proper percentages, and times of exposure were arrived at. This work had been previously done by Professor Hottes, and the tables just preceding show the periods used, the percentages employed being 1-1/2, 2 and 2-1/2. In general, the best results seemed to come from the 2% solution.

The following remarks enter into consideration with more or less effect upon the results. In all cases the exposures were started at about eleven o'clock in the morning to insure bringing as great a number of division stages as possible under the influence of the antipyrine. Of course it is to be remembered that some time is required for the anyipyrine to reach the center of the root after the latter has been immersed, and also that some antipyrine remains in the root after it is transferred to tap-water for regeneration. The effect of this last is frequently shown in divisions which take place during the regeneration period.

In the case of material killed immediately after removing from the antipyrine, and also when the regeneration period was only five hours, about one centimeter was cut from the tip and

fixed.* In the cases of longer intervals for regeneration, a record was made of the length before exposure to antipyrine, and the measurement was again taken at the end of the regeneration period; the amount of growth in the meantime being taken into account in cutting off the portion to be fixed, so that the region previously exposed to antipyrine was included. When growth during the regeneration period amounted to eight or ten millimeters, the exposed region was in this case pushed back into the zone of elongation where the protoplasm is only a peripheral layer, and hence not much chance is offered for a study of the structure of the cytoplasm.

After twenty-four hours fixation, the material was washed, dehydrated, and transferred to 43°C. parrafin by the use of chloroform, the paraffin being dissolved in the chloroform with low heat. After the chloroform had been evaporated off, leaving the material in pure 43° paraffin, transfer was made to 52° paraffin in which the material was embedded. Sections were cut 7 to 10 microns thick and stained in the Flemming triple stain; the method followed being similar to that used by Mottier** in his study of division in pollen-mother cells.

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* The following two solutions were used for killing and fixing, and if any difference in the results was noticed it was that the staining capacity of the spindle fibres especially, was best brot out with the Flemming solution.

Flemming's solution,

osmic acid	1 gr.
chromic acid	3.6 gr.
acetic acid	24 cc.
water	432 cc.

Chromo-acetic solution,

chromic acid	1 gr.
acetic acid	4 gr.
water	1000 cc.

** Mottier- "Bieträge zur kenntniss der kerntheilung in den Pollen mutterzellen einiger, Dikotylen und Monokotylen" - in Strasburger's "Cytologische Studien" 1897.

EFFECT ON THE CYTOPLASM.

The striking appearance of the cytoplasm in material treated for an hour or more with a two per cent soultion of antipyrine can not escape the attention on the first examination. The result is a decidedly alveolar structure, which varies in prominence with the time of exposure and strength of the solution. The following table shows the conclusions drawn from two or three sets of roots treated with the three different strengths of solution:-

1-1/2%solution. Without regeneration.

1/2hr.	exposure,	Alveoli rarely present
1 hr.		"
1-1/2hr.	"	Alveoli present but not well developed.
2 hr.	"	Alveoli present and better than in above.
2-1/4hr.	"	"
2-1/2hr.	"	Alveoli present and fairly developed.

2 % solution. Without regeneration.

1/2hr.	exposure,	Alveoli formed not well developed.
1 hr.	"	Alveoli formed and more prominent than in above.
1-1/2hr.	"	Appearance about the same as above.
2 hr.	"	Alveoli good to excellent.
2-1/4hr.	"	Alveoli excellent.
2-1/2hr.	"	Probably no better than above.

2-1/2 % solution. Without regeneration.

1/2hr.	exposure,	Alveoli present and fairly developed.
1 hr.	"	Alveoli good.
1-1/2	"	"
2 hr.	"	"
2-1/4hr.	"	Alveoli good but without thick walls.
2-1/2hr.	"	Alveoli good but flaky around axis cylinder.

Individual variations are present, so that in some instances, probably depending on the activity of the root, good alveoli were obtained from material exposed for only one half hour to 2% solution. Fig. 1 is a microphotograph showing the conditions obtaining in a single region of the division zone. While the photograph does not show the detail clearly, the excellent alveolar appearance is brought out. Fig. 2 is a microphotograph to show the appearance of the cytoplasm after regeneration. This will be referred to again. Figs. 3 and 4 are drawings from the same regions as that from which the first microphotograph was made. Fig. 3 is from the epidermis on the right inward, and except for two or three rows of cells between the left edge of Fig. 3, and the right edge of Fig. 4 , the two figures together present a continuous view of the appearance of the cytoplasm from the epidermis inward to the center of the axis cylinder.

In the epidermal cells the cytoplasm is dense and granular, with numerous small cavities, immediately within this layer the alveoli gradually grow larger for some distance inward, then decrease in size until the last two or three layers surrounding the axis cylinder are reached when the alveoli give place to a densely flaky structure which tho less densely, is continued into the axis cylinder. The cells

of the root cap contain larger alveoli than those of the root itself immediately above it, and from the tip of the root upward the alveolar condition gradually becomes more prominent, the alveoli gradually becoming larger, until in the stretching zone, by coalescence, they form vacuoles. Thus within one organ, the root, we have the cytoplasm of different regions responding very differently to the same stimulus, both from the outer layers inward, and from the tip upward.

Turning now to the regeneration material, the following table shows the conditions of the cytoplasm after five hours regeneration from previous exposure to a 2% solution:-

1/2 hr.	previous exposure,	No alveoli, cytoplasm flaky.
1 hr.		"
1-1/2 hr.		"
2 hr.	"	Some alveoli but those disappearing.
2-1/4 hr.	"	Some alveoli still present.
2-1/2 hr.	"	Few alveoli still present.

After twenty-four hours regeneration the alveoli have entirely disappeared except in the high per cents. In one case in which the previous exposure had been two hours in a 2% solution, and the growth seven millimeters during the regeneration of twenty-four hours, thin walled shells were still present to indicate an alveolar structure. It might be added that the indications of the alveolar condition were not at all well marked, and further it will be noted that with each succeeding division the amount in any cell is halved, so that with division sufficient to produce seven millimeters growth the amount

remaining in any one cell must have been very small, and we should have expected that by that time the plant would have become acclimated to so small an amount of antipyrine.

It seems as tho in regeneration the alveoli break down and from their disintegrated walls results a flaky cytoplasm. This flaky condition is distinctly different in appearance from the granular condition; the flakes in themselves being peculiarly transparent. The variation in the effect upon the cytoplasm in different regions, and the disappearance of alveolar structure on regeneration favors the view that there is no definite structure for cytoplasm; but that the structure varies with the conditions.

EFFECT ON THE KINOPLASM.

Fully as prominent as the change produced in the structure of the trophoplasm is the effect of antiphyrine on the kinoplasm of the spindle. Material exposed for one-half hour to a 2% solution shows this; and the effect continues for some time after the material has been transferred to tap-water for regeneration. This is shown in cases of distorted spindles which undoubtedly had their origin after the regeneration period commenced. Fig. 10 shows typically what this effect is. It is evident that the spindles in both the upper cells are too long to stand in the normal vertical position, and in the lower cell the mitotic figure only remains vertical by having its lower pole thrust forcibly down into the pocket-like lower end of the cell. Furthermore , while the layer of trophoplasm in every case lies between the chromatin mass at the end of the spindle and the cell wall itself, yet the shape of the chromatin masses shows that there is some force which tends to fit them into the corner

or pocket in which they lie. In the case of the upper end of the spindle in the lower cell, this force is flattening the chromatin mass out and applying it to the upper wall. Also, where portions of individual chromosomes are still visible these free ends are being pressed toward the adjacent cell walls. In other words, as illustrated especially by the middle and lower cell, it is as tho some force between the chromatin masses were endeavoring to separate them as far as possible. That the spindle is this force is shown by the fact that it is too long to assume its normal position and in its continued growth, has glided around in the cell, pushing before it the chromatin mass. The wavy appearance of the fibres in the middle cell of Fig. 10 and also in the upper right hand cell of Fig. 6 shows that the further increase in length of the spindle is hindered by its poles reaching the diagonal corners of the cell, the growth of the fibers still continues, and, due to the resistance, may become wavy. Fig. 24 shows a spindle the upper end of which has forced its chromatin mass around. Here the spindle is beginning to disintegrate, but the fibres remaining show the wavy appearance. Thus by the antipyrine the spindle fibres are stimulated to an increased growth in length and this elongation of the spindle forces the masses of chromatin apart. These are not exceptional cases, but rather the rare exception is to find a normal spindle, except in the region of elongation where it would require an exceedingly long mitotic figure to reach the entire length of the cell.

In other cases, later stages as indicated by the loss of identity of the chromosomes, no recognizable spindle remains, but in its stead a mass of fibres crossing each other in different directions, but at

the same time straight, or curved in a way to indicate a stress and an elongating tendency . Figs. 7 and 9, and 11 illustrate this. Occasionally a spindle is found which still occupies a vertical position in the cell, but its bulged appearance gives evidence of the elongation of the spindle fibres. In the upper right hand cell of Fig. 15 the original position of the spindle is shown by the present densely granular area, and from this it can be seen that the spindle was much rounded.

Now that we have seen that the fibres grow in length, and that that growth is a forcible one, we may ask what is their origin and where is there center of growth if such exists. If the spindle fibres originate from nuclear reticulum, would it seem probable that more than the normal amount of spindle-fibre material could be produced? That is, if the nuclear reticulum goes to form the spindle fibres then when that amount is used up, how can more spindle-fibre material be formed, as is the case under antipyrine stimulation, when both the number and length of the fibres is increased? It may be replied that not all the nuclear reticulum is used normally and that by stimulation the reserved store is called into use; but it is evident that a nucleus many times larger than that in Vicia Faba would be required to contain nuclear reticulum sufficient to produce the amount of spindle fibres shown in Figs. 9 and 13, when it is remembered that the drawings show only the fibres in a single radial plane of the spindle. In answer to the hypothesis that the fibres arise from both cytoreticulum and nuclear-reticulum, it seems sufficient to ask how can a cytoreticulum take part in their formation when no cytoreticulum exists? For none is visible in the cytoplasm

of this material under the conditions to which it was subjected.

Leaving for a time the question of the origin of the spindle fibres, we will turn to that of growth. Spindle fibres grow. Further, we do not find isolated short bits of fibre out in the cytoplasm, or in the region of the nuclear membrane, growing in length and lying in the direction of the future spindle. That is, the spindle does not originate either out in the cytoplasm or within the nucleus, as a lot of short rods which later grow in length, and remains parallel, come eventually to have their ends drawn together, thus giving rise to a spindle. On the other hand, short fibres are frequently found, upper right hand cell of Fig. 6, extending from the pole of the spindle outward; that is, growth of the spindle-fibre is from that point as a center which is later to become a pole of the spindle. Whether its growth is by additions at the free end or additions at the basal end, is the question to be taken up next. If growth took place at the free end, then spindle fibre formation would be something comparable to a crystallization process. A center of growth, whatever that might be, would have to exist at the free end of every spindle fibre and by a precipitation,possibly, of something in the cytoplasm, elongation might take place. But when the free end of a fibre comes in contact with the cell wall, for instance, growth in length does not give place to growth in thickness; but rather the fibre continues to elongate without any evident thickening. Further, the mere fact that a spindle has its fibres drawn together toward a common center at each end suggests that they arise at a common center; for if they had their origin in the cytoplasm or in the nucleus and grew in haphazard directions, what force

would guide them to a focus at the poles? We seem compelled to conclude then, that spindle fibres have their origins at the poles of the spindle as centers of radiation, and their growth is by addition of matter at their basal ends, something as the mercury column in a thermometer increases in length by additions from below.

So far nothing has been said of any distinction between mantle fibres and central spindle fibres. It is generally assumed now that the central spindle fibres, which pass from pole to pole exert a push tending to separate the ends of the spindles; but a difference of opinion exists as to the action of mantle fibres. The concensus of opinion is, however, that the movement of chromosomes is brot about by these fibres, hence the discussion resolves itself into a question of whether the chromosomes are pushed or pulled by these fibres. The first objection to the theory of the shortening of the mantle fibres has been raised many times,- namely that if the chromosomes are moved in this way the fibres themselves must shorten to nil; but if this takes place, the best observation does not seem to confirm the existence of any thickening of the fibres, which would be expected to result from a shortening in length. It might be answered that the chromosomes need not be brot any closer to the poles than is necessary to make contact between them individually and so secure a fusion of the chromatin matter; but looking at such figures as 8 and 10 gives one an entirely different view of the matter. It will be noticed that wherever the ends of the chromosomes project still from the general chromatin mass, that is, in cases in which only one end of a chromosome has become fused with its fellows and the other still remains free, this free end is in such a position

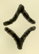
as to indicate that it is being shoved away from the axis of the spindle. Such a force may readily be conceived as due to the growth of spindle fibres radiating from the opposite pole of the spindle, and having their distal ends applied to these free ends of the chromosomes. No other assumption seems applicable, and Fig. 8 shows a case in which such spindle fibres could actually be seen. In the two upper left hand cells of Fig. 35 it is evident that the chromosomes have no definite arrangement and this haphazard condition is due to the energetic growth of fibres which are pushing the chromosomes in all directions, or at least this must have been the case while the spindle was yet functional and had not commenced to disintegrate; for had the function of the spindle been performed normally, the polar masses would have been formed in the proper manner. Under the present condition however, it is evident that the separation of the chromosomes into polar groups can not take place and the probable outcome would have been the re-fusion of the whole chromotin mass into one giant nucleus.

Returning now to the method of chromosome movement it can not be said then, that they are pulled to the poles by the mantle fibres, and that later these masses are pushed apart by the central spindle fibres, for in Fig. 8 we have a forcible separation of the masses as shown by the diagonal position of the spindle, and at the same time there is a pushing of the chromosomes as already discussed. Why then, shall we make a distinction between mantle and central spindle fibres? It may be replied that proving the non-existence of such a distinction in Vicia Faba is treating only a single instance; and to this again it may be responded, will not the theory of elongation of all the fibres explain the facts observed in any case, just

as well as will the theory of contractility of mantel fibres, and elongation of central fibres? To this may be added any argument coming from plausibility, namely, that it is a simpler and apparently just as applicable a theory to suppose that all the fibres have the same origin and the same characteristic of growth, and that in the origin, some are not especially destined to remove chromosomes, nor others to become central spindle fibres; but in their growth toward the equator from the poles, some come in contact with chromosomes which they shove ahead in their growth and others not coming in contact with chromosomes, grow on past the equator toward the opposite end of the spindle.

Just why a spindle is elipsoidal in shape, does not seem easily explained; but in view of the elongation of the spindle fibres, as already demonstrated, something of the causes may be gotten at. If under normal conditions spindle fibres are produced only on the inner side of each region, later to become a pole of the spindle, these fibres would meet at the equator and the figure would be that of two cones, base to base. To convert such a figure into an ellipsoid, it is only necessary to introduce some resistance to the separation of the poles of the spindle which will then cause the spindle fibres to bulge out as their growth continues. The question then is, what would serve to offer such a resistance? Looking at such a case as is shown in Fig. 8, we have seen that the diagonal position of the spindle is due to its elongation . At the same time we see that a layer of cytoplasm remains between the chromatin masses and the cell walls. This in itself might perhaps indicate that the viscosity of the cytoplasm offers some resistance to the movement of

chromatin masses, and the whole bulging of the spindle would seem then, to be a question of the excess of the resistance due to viscosity over the rigidity of the spindle fibres. Thus, it does not seem impossible to conceive of the resistance of the cytoplasm in virtue of viscosity, being greater than the rigidity of the spindle fibres, and hence causing a bulging of the spindle, yet at the same time; within the spindle this elongation of the spindle fibres may be sufficient to move the chromosomes.

Returning again to the question of bulging as resulting from some resistance to the elongation of the spindle , the question arises, why should the fibres not bend inward, as well as outward, and the result be such a figure as  , rather than an ellipsoid? It seems permissible to assume that since the fibres forming the core of the spindle are perpendicular to the equatorial plane of the cell, they will be the first ones to make continuous connection between the poles of the spindle, since fibres oblique to the equatorial plane must grow farther in order to come in contact with their fellows from the opposite side. Were such a core as this formed, it seems as tho it would in itself, resist any tendency of the fibres to bulge inward, and hence the bending would have to be outward, and would result in the ellipsoidal spindle.

To sum up what we have said so far on spindle formation we have first the formation of spindle fibres on the inner edge of regions later to become poles of the spindle. These fibres are all similar in origin and characteristics of growth, and as they grow toward the equatorial plate some come in contact with chromosomes, which they shove ahead to the opposite pole and others meeting no resistance, probably grow on thru toward the opposite pole. The viscosity of

the cytoplasm.is the obstruction offered to the elongation of the spindle, and this causes the bulged or ellipsoidal shape.

The fact that chromosomes are not pushed outside the spindle, as they are forced along to the poles, may be explained by the existence of so large a number of spindle fibres that these in themselves make it impossible to force the chromosomes out; but at the same time, being parallel with the direction of travel of the chromosomes, offer no great resistance to the passage of the latter in the proper direction.

Fig. 9 is a case in which the spindle has been under the action of antipyrine probably since it was in the metaphase stage. The growth of the spindle fibres has been exceedingly energetic, so much so, that it seems that where the two ends of opposing fibres come in contact (at the equator), they have slipped and passed by, going directly across to the opposite side of the spindle, and many of them,by continued growth after coming in contact with the cell wall, have become bent in different directions. The chromosomes had evidently reached the poles before this growth of the fibres was at its maximum, otherwise they would have been pushed in all directions as in the two cells of the upper left corner of Fig. 35. In such cases as are represented by this last figure,it is clear that normal daughter nuclei can not result.

Figs. 18, 19, and 17 form a series in which it might appear that division by simple constriction, amitosis, is taking place. It will be noted that no cell wall appears between the nuclei, and at first sight, not having earlier stages on which to base conclusions, it might be said that anitpyrine has induced amitosis where mitosis

normally exists, and a coenocytic cell is the result. Fig. 16 however, lends some doubt to such an hypothesis, since we have here a binucleated cell, the two nuclei of which however, are in metakinetic, or early anaphase stages of division. Fig. 5 is a stage in which the spindle activity probably ran their cycle before their purpose was entirely accomplished, and began to disintegrate before all the chromatin matter was moved to the poles. Whatever the cause, the fact remains that a chromatin bridge connects the two polar masses of chromotin and from this we get some clue as to the origin of the pseudo-amitotic figures. Suppose that each of the chromatin masses should pass on to the resting stage; a nuclear membrane would be developed around each mass; but no force would be present to break the chromatin bridge. On the other hand, from the fact that nuclei tend to occupy a central position in the cell, the interval between the daughter nuclei would probably be shortened and in this process, part of the chromatin of the bridge would be absorbed by the polar masses. Thus we would have such a condition arising as is shown in Fig. 18; and Fig. 19 is similar except that either the original chromatin bridge was larger, or in their movements toward the center, the two masses came into closer contact. In such cases as Figs. 7, 9, 11, and 14, it is evident that no cell wall will ever be laid down between the poles of the spindle, and hence if the two chromatin masses round up quickly, two nuclei will be formed in each cell. The last statement implies that a fusion of the two masses might take place if this rounding up , and taking on of the resting stage did not occur quickly. It appears that if contact be made before both masses have assumed the resting stage, and before a nuclear membrane has been formed, then, depending on the complete-

ness of the fusion, a giant nucleus, or a pseudoamitotic figure may result. That is, it seems that after the nuclear membrane has been formed no further fusion of the masses takes place; they remain in their isolated or partly united condition; but so long as this nuclear membrane is not formed, fusion may take place and the chances for its occurrence are aided by the tendency of the masses to move towards the center of the cell.

Similar conditions obtained from mitotic figures which were placed under the influence of chloral hydrate have been studied by Wassielewski[*] who interprets the phenomena as normal amitosis, which by the action of chloral hydrate has been induced in the place of normal mitosis.

Nemec[**], however, in the same number in which Wassielewski's last article appears, describes the same conditions, and while his ideas are evidently not altogether the same as those intended to be conveyed by this article, yet he sees the conditions as they really are, namely, abnormal variations of mitosis, and not at all due to a constriction of the nucleus.

While the immediate cause of the dumb-bell shaped nuclei is the existence of a bridging chromatin band, the ultimate origin is in the abnormal spindle. On account of the increased stimulation in spindle fibre production induced by the antipyrine many more fibres may be produced on one side of the spindle than on another, and along with this may come also an unequal distribution of fibres to the chromosomes, this last resulting in the failure of some of the chromosomes to be moved to the poles. The result is such a bridge as is shown in Fig. 5.

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[*] Jahrb. f. Wissen. Bot. Bd. 38; Hf.3.

[**] " Bd. 39; Hf.4

The relation of fibres to the cell plate is brot out in the two cells to the right in Fig. 15, in which it is evident thaton account of a lack, either of uniform distribution or uniform growth of the fibres on the two opposite sides of the cell plate, the irregularities shown in the drawing have been produced. That the fibres are in a state of stress is shown by the flattened state of the nuclei. We may assume that at one point in the cell plate more force is being exerted from the side of the upper pole than from that of the lower one; at another region the opposite may be true, and as a result, in the first case the plate is bulged toward the lower pole and in the second case toward the upper. This inequality of force may result from an inequality in the number of opposing fibres at any region of the cell plate, or it may arise from an inequality of the elongation of the fibres striking the opposite sides of any definite area of the cell plate.

Just what the origin of the plate is does not seem clear; but that its substance is closely fused with the substance of the spindle fibres, seems certain from the appearance of the two cells to the right in Fig. 15. For in this case, if union of the cell plate and spindle fibre substance did not exist, then the spindle fibres in their unequal growth would not affect the plate as they do; but simply slip thru it like wires thru the meshes of a screen. This intimate relation of the spindle fibres to the plate is further shown in Fig. 20, and in the left cell of Fig. 15, in both of which only a corner of the mother cell has been cut off. Such cases as these were not exceedingly common; but were of sufficiently frequent occurrence to warrant their being undoubtedly due to antipyrine.

In the case of Fig. 20, and the cell to the left in Fig. 15, it appears as tho the cell plate had been partly laid down on the right side in both cases, but that due to a turning of the spindle which occurred about this time, or at least before the laying down of the cell plate was completed, the mitotic figure came to occupy a horizontal position, and in this position the formation of the left half of the cell plate was completed. Thus we have another demonstration of the intimate relation between the spindle fibres and the cell plate itself. To the right in the lower cell of Fig. 15 a bit of phragroplast remains at the edge of the cell plate. This figure corresponds undoubtedly with many of the figures of Neméc and Wassielewski, and it may be that their similar figures from chloral hydrate material were produced by the stimulating effect which that poison probably had, before its retarding effects set in.

In the case of Fig. 20 it appears that the spindle was still in practically a normal position when the right edge of the plate became associated with the wall of the mother cell, but before the left edge had become attached, the twisting took place and by this twisting the present shape of the dividing wall and also the relative position of the daughter nuclei is explained. In the case of the cell to the left in Fig. 15, it appears that before any union of the cell plate to even the right wall of the mother cell took place, a slight turning occurred which caused the point of attachement of the cell plate, when union did take place, to be farther up than the middle of the mother cell wall. Even after this attachment took place movement of the spindle continued until its axis became horizontal, and conditions similar to those of Fig. 20 resulted.

The discussion of the origin of spindle fibres has been left until their action and effect has been shown. It has already been hinted that they seem to be emanations from two opposite centers in the cell and that they grow by the addition of substance at their basal ends. That is, it seems that the points called the poles of the spindle, are the centers of growth, and that elongation of the spindle fibres takes place by the addition of material at their basal ends. It would appear then, that some substances, antipyrine for example, accelerate the production and growth of spindle fibres; that is, increase that action by which some substance, kinoplasm, is either removed bodily from the cytoplasm, or is perhaps constructed out of the trophoplasm and is converted into spindle fibres. The idea of an enzyme being the origin of spindle fibres was suggested by Carnoy in 1885, but his theory has received little attention since then. Whether or not such a theory seems probable, it nevertheless fits in easily with the conditions here described. The objections to the theories of origin from nuclear and cytoreticulum have been discussed and proof has been presented for the statement that all spindle fibres elongate, and that forcibly; it then remains to show whether the growth is from the poles of the spindles as centers, or is by the addition of matter at the free ends of the fibres. This has been touched upon before but we may now look at it again. There is little doubt but that when a spindle arises the polar radiations are its first indications; these radiations later meet at the center and the resulting figure is the completed spindle. This statement in itself suggests that growth is from a common center, for we have assumed that the origin is from a common center. Furthermore, if

growth took place by the addition of material at the free end of the fibre, then to keep the fibres all drawn together in a focus at the poles, we would be compelled to assume that some substance is present at the poles to perform this office. On the other hand, assuming growth to take place from the basal end, the poles themselves become the centers of growth, and act to hold all the basal ends of the fibres together, while the free ends radiate out. On the whole, this last hypothesis seems the more plausible and finally, to explain the process of growth, the enzyme theory, or a theory closely related to that, seems at least tenable.

THE EFFECT ON THE CHROMATIN.

When we direct our attention to the chromatin we notice other abnormalities. Abnormalities, which again in this instance seem only the normal phenomena in a more marked degree. The cause of these peculiarities may lie ultimately in the spindle fibres which, by their exertion of abnormal stress, have pressed the chromosomes more closely together at the poles than is normal, and thus has arisen the characteristic densely packed polar masses. Fig. 6 shows three examples of what is referred to. The striking peculiarities in appearance are more prominent as this dense mass is converted into a resting stage, and by examining these stages we are able, it seems, to see what the steps are in the normal conversion of the late anaphase into the resting stage. Thruout this whole investigation all the interpretations of normal conditions, as the elongation of all the spindle fibres, etc., have arisen from the interpretation of the abnormally pronounced conditions as they are occasioned by the accelerating effect of the antipyrine. That is, the

conditions here described as resulting from antipyrine are merely abnormally pronounced normal conditions, and what occurs in the normal activities of the kinoplasm, for instance, is simply a repetition of these same phenomena, only in a less pronounced degree.

The first apparent change in the solid mass, as it is converted into the resting stage, is the appearance of holes, small at first, but gradually growing larger and more circular. Fig. 11 is such a stage in which the clear areas represent the holes. The real nature of what we have called holes is better seen when we examine stages a little later, in which the clear areas are larger. If we make drawings of the chromatin mass from a plane, for example above the center, at the center, and below the center of the chromatin mass, as in Fig. 26, we find that these drawings do not at all coincide one with another,and as the focus is changed from one plane to another we can see the gradual change in appearance. That is, starting with the upper focus as shown at "a" and focusing downward, thru the mass, "b" gradually comes in view, and then "c". The two upper clear areas to the right in "a" change in appearance, and become the two upper clear areas to the right in " b", but these are not the same clear areas that are shown in the upper margin of "c". Also, the largest clear area at the lower left margin of "b", which is not present in "a", becomes the lower clear area of "c". We see then, that this chromatin mass is a shell pierced with holes and having cross bars of chromatin traversing its interior in different directions. A representation of such conditions is intended to be shown in Figs. 21, 22, 23, 24, and 25. By producing more holes in the wall of the shell, and by the enlargement of those already present,

and also by the reduction in the diameter of the internal chromatin bridges, the normal resting stage of the nucleus seems to be attained. Along with these last changes in structure goes the change in staining capacity. The chromatin begins gradually to take the gentian violet rather than the saffranin.

Concerning the origin of the cavities within the nucleus, it seems quite clear that they arise spontareously; that is, vacuole like spaces seem to appear which gradually increase in size and coalesce with their neighbors, giving rise to the transversal bridges when the common walls of the contiguous vacuoles are not entirely eliminated, and producing holes in the wall of the shell where these vacuoles break thru the surface.

Frequently small globular masses of chromatin are seen out in the trophoplasm. At first it was a question as to whether or not they really were chromatin masses; but very close examination revealed a connection between them and the general mass, as is shown in the cell to the left in Fig. 6, also the lower chromatin mass of Fig. 14 and the upper mass of Figs. 22 and 25. The origin of this extranuclear chromatin does not seem clear, but by examining stages of development as shown in the cell to the left in Fig. 6, and the lower mass of Fig. 25, it is evident that the little ball at the end of the fine thread arises as a knob on the surface of the larger chromatin mass, and, as it passes out from the mass, the connecting thread gradually becomes smaller. The fine thread projecting from the lower surface of the lower mass of Fig. 25 is without a knob on its distal end and is, incidentally, the longest one of the threads projecting from the mass. This points to the origin of the extranuclear mass as being traceable to the breaking off of the knobs.

It further appears that the knobs arise near the surface of the large chromatin mass and pass outward into the trophoplasm, gradually drawing out the connecting bridge into a fine thread. How the knobs appear in the first place is not evident. Perhaps they come from the protruding ends of chromosomes. Some chromosomes may not fuse entirely with the general mass, and the free ends may be sloughed off in the form of extra-nuclear masses of chromatin. We may ask also, why these chromatin globules pass directly out from the chromatin mass, keeping the bridging thread taut. Is the thread itself stiff, or is some force at work pulling it out?

In this connection it may be interesting to ask what differences will result in the number of chromosomes when such a nucleus as is shown in Figs. 18 and 19 passes thru the metakinesis stage. The number of chromsomes in the normal cell is not constant, and the number which would arise from the division of these dumb-bell shaped nuclei can not be placed at twice the normal number, for the fact of their being dumbbell-shaped depends on the existence of a chromatin bridge; and this in turn varies in size, depending as we have seen, in one case on the number of chromosomes not moved to the poles, and hence forming the connecting bridge, and in the other case on the completeness of the fusion of the polar masses, in case they move together as a result of the failure to lay down a cell plate. That giant nuclei do exist is true, but their occurrence is not very frequent.

EFFECT ON THE NUCLEOLUS.

One half hour exposure to a 1-1/2% solution of antipyrine is sufficient to cause the disappearance of the hof in all regions of the periblem except the very tip of the root. In the latter region the nucleolus may remain normal thru even two and one half hours exposure to a strength of 2-1/2%. In the same way, the nucleoli in the axis cylinder do not seem to be affected by the stimulus. With the dissapearance of the hof, the sharply rounded outline of the nucleolus gives place to an irregular contour. The size of the nucleolus becomes much greater than normal, but that the substance of which it is composed is not as dense as it was, is easily concluded from the paler stain which it shows. It is as tho the once dense and rounded body had, with the disappearance of the hof, begun to rapidly diffuse out in all directions. The process might be compared to dropping a bit of dry gelatin into water. Its distinct outline disappears with the absorption of water, and at the same time the whole mass swells to a greater size. Why did this diffusion of nucleolar matter take place? Evidently it is correlated with the disappearance of the hof, that is, the removal of the clear area from around the nucleus.

Hottes* has confirmed quite conclusively, it appears, Strasburger's theory of the function of the nucleolus, namely, that it is a reserve material rather than an accumulation of waste products of the metabolic processes of the nucleus, or an accumulation of chromatin to be given out again at the development of the spireme stage. Whether

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*Strasburger. Reduktionsteilung, Spindelbildung, etc., im Pflanzenreich. p.127.

this reserve material is of the nature of kinoplasm, or is simply trophoplasm accumulated within the nucleus, is another question. That it disappears when the cell is compelled to work hard has been demonstrated* , and on this fact hinges the statement that it is a food supply rather than a waste product or a chromatin accumulation. The hof has been explained as the zone of diffusion outward of nucelolar matter from the nucleolus itself; that is, the reserve material of which the nucelolus is composed has been precipitated in some way, and to be of use in the cell activities must be re-dissolved. The re-dissolved material is of necessity different from the nucleolar matter itself, and does not take the chromatin stain as the latter does. With increased cell activity, caused for instance by compelling the root tip to grow under pressure, as Hottes* has done, the hof disappears; that is, the reserve material is taken up as rapidly as it is given off, and thus has no chance to accumulate. We have already seen the stimulating effect of antipyrine, and the disappearance of the hof may well be taken, it seems, to be a result of this same increased activity which uses up the dissolved nucleolar matter as fast as it is formed. We would naturally expect then, that the nucleolus would become smaller in size as this process of dissolution and removal takes place, and measurements were made to determine this. First, it is evident that since the size of the nucleolus is somewhat in correlation with the size of the nucleus, and since the latter varies in size and shape, especially in this material, it will be necessary to compare the size of the

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*Hottes. Unpublished work.

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nucleolus to that of the nucleus under different conditions of treatment, if we wish to get at the effect of such a treatment, rather than to compare simply the sizes of the nucleoli. The next factor is the irregularity in shape of both the nucleolus and the nucleus. The irregularity in shape of the nucleolus, resulting in the diffusion outward of its substance in all directions, has been mentioned, and the irregularity in the shape of the nucleus will be taken up later. Because of this irregularity it will not be possible to focus on a nucleus or nucleolus and say that the drawing thus made represents exactly the relative size of the nucleolus and nucleus, for in a plane just above or below the one we choose, the size and shape may be very different; and had the nucleus been viewed from a different direction, that is, had the root been ~~plucked~~ from a different side, the shape would have been still different. We may fairly expect, however, to get some idea of the facts as they exist, even by this process, if we are careful to choose a plane of focus which shows the body which we are examining in about its average size. This was attempted as carefully as possible with good material from exposures of one half and two and one half hours respectively, to a 2-1/2% solution. Fifteen nuclei were drawn from each series of material, and separate from these on the same paper, the nucleoli belonging to these nuclei were drawn. To determine the area of these drawings the simplest method, on account of their irregularity in shape, was to cut out and weight the figures of the nuclei and nucleoli respectively for each series. From this we get a comparison of the cross-sectional areas of the nucleoli and nuclei. In the case of the one half hour exposure the ratio of the cross-sectional area of

the nucleolus to that of the nucleus is 1:4.1, and a similar comparison for the two and one half hour material gives the ration 1:4.3. It is of course, understood that the ration of a cross-sectional area is not the ratio of the volume; but in the above two cases the difference in results is so small that it could easily fall within the limit of error, for the possible error is of necessity very great. It would appear then that after the first effect upon the nucleolus, namely the sudden disappearance of the hof and the assumption of the much larger and more irregular form, the subsequent exposure produces no noticeable change. A striking peculiarity however, which is noted as one examines the two and one half hour material for nucleoli, is the occasional occurrence of nuceli with no sign of a nucleolus, or at least nothing that can be called definitely a nucleolus, and not a heavier chromatin mesh. Furthermore, the number of such cases is greater in two and one half hour material than in two and one-fourth hour or two hour material. Thus, to find the fifteen nuclei on which to base the calculations for the two and one half hour series, it was necessary to omit many which appeared either to have no nucleoli, or only very slight and indistinct traces of such. This is not true of the half hour exposure. In attempting to make sure that each was a clear case, some undoubtedly clear cases were omitted. Another point also, which will help us out in the end, is the staining of the nucleoli in this two and one half hour material. In no case is it distinctly red, and of the characteristic clearness; but rather, the stain is paler, sometimes yellowish, and an observer is led to feel from the appearance that the structure is not nearly so dense as normal, and

less dense than in the one and one half and two hour exposure material. Radiating out from these irregular nuceloli and connecting with the chromatin network on the surface of the nucelus, are a great number of thread-like bridges of nucleolar matter. Their appearance suggests that the linin network within the nucleus has been converted into a path over which the nucleolar matter is passing to the periphery of the nucleus. This dissolved nucleolar matter undoubtedly passes out of the nucleus; and if it accumulates more rapidly than it can diffuse thru the nuclear membrance, a swelling appears on the side of the nucleus, that is, the bulges giving rise to the irregular appearance of the resting nucleus, are caused by the accumulation of dissolved nucleolar matter which distends the nuclear membrane. Frequently very small bulges of the nucelolar membrane can be seen pushing out from the meshes of the peripheral chromatin network. Fig. 27 is a nucleus from the tip of the root, showing the nucleolus with a well defined hof; the nucleolus itself being dense and sharply outlined. Fig. 28 is a nucleus from the same section, but farther up. The size and irregularity of the nucleolus, and the absence of a hof are noticeable. Fig. 31 is a nucleus from material exposed two and one half hours to a two and one half per cent solution. No nucleolus is visible here. Figs. 32 and 29 are what have been interpreted as stages in the final degeneration of the nucleolus. Fig. 30 shows the fine lines of chromatin matter radiationg out from the nucleolus to the nuclear membranes. Figs. 33 and 34 show the irregular, or lobulate, appearance of the nucleus which is correlated with the dissolution of the nucleus.

SUMMARY.

The following general results and conclusions seem warranted. The structure of the cytoplasm depends on the external environment to which it is subjected, and also upon its own inherent characteristics, which latter may cause different results to obtain in different tissues closely associated in the same organ, and placed under the same conditions of environment. All spindle fibres are the same in origin, and have the same characteristics of elongation. They all originate at the poles of the spindle as centers of growth. As they grow toward the opposite pole of the spindle, some come in contact with chromosomes which they push ahead, and others, meeting no obstruction, pass on freely to the opposite end of the spindle. By the stimulating effect of antipyrine an excessive growth in the number and length of spindle fibres may be induced and in virtue of the abnormal spindle thus formed, there may result a failure to lay down a cell plate. Pseudamitotic figures may arise by the failure of all the chromosomes to be moved to the poles, thus leaving a connecting bridge. It has been suggested that they may also arise by the fusion of normal polar masses in those cases in which no cell plate is laid down to keep them apart. Origin by the first method seems undoubted; but the proof of the second lies chiefly in the observation that in those cases in which no cell plate is formed, and normal polar masses exist, they lie very close together. By continuing the action of antipyrine these binucleated cells will, with the next division, become quadrinucleated cells. If the cell plate has become attached at one side of the mother cell wall, then

by a growth of the spindle sufficient to cause it turn diaognally in the cell, the cell plate may be bent in such a way as to finally cut off only a corner of the mother cell. By the action of antipyrine the chromosomes seem to become very closely packed in the polar masses, and in the conversion of these into resting stages numerous cavities arise inside the mass giving rise to a spong-y structure. These vacuoles break thru the surface, and by the coalescence of those within, a resting nucleus is formed, with a net work of chromatin on the outside, and traversal threads within. With the increased activity of the cell the hof disappears from around the nucleolus, and the latter becomes irregular in shape, and larger. As it becomes larger it also becomes less dense, and by means of the radiating arms extending out from it, its substance is conveyed to the periphery of the nucleus. That the linin is the path along which this passage takes place seems probable. This process may be carried on so far that no well defined nucleolus remains, but in its place only a net work, scarcely if at all distinguishable from the chromatin network. Extra-nucleolar chromatin exists in the form of small globules. Their origin does not seem very certain.

EXPLANATION OF FIGURES.

With the exception of the microphotographs, all the drawings were made with a camera lucida. In most instances, as can be seen from the magnification oil immersion objectives were used, either a Bausch & Lomb one-twelfth or a Spencer two mm. The low power drawings were made with a Spencer four mm. and sixteen mm. objectives.

In the following table, "expos." refers to the length of exposure in antipyrine, and "reg." to the length of the period of regeneration; the strength of the solution being indicated by the per cent.

Fig. 1. Microphotograph to show alveolar cytoplasm

2-1/2 expos.; 2%

Fig. 2. Microphotograph to show formation of granular cytoplasm by the breaking down of the alveolar walls. Some alveoli still show to right.

1/2 expos.; 24 reg.; 2%

Fig. 3. Alveolar cytoplasm, epidermis to right.

2-1/2 expos.; 2% x 795

Fig. 4. Alveolar cytoplasm, axis cylinder to left

2-1/2 expos.; 2% x 795

Fig. 5. Chromatin bridge

2 expos.; 2% x 1890

Fig. 6. Distorted spindles

2-1/4 expos. 2% x 1890

Fig. 7. Disintegrated spindle Extra-nuclear chromatin

2-1/4 expos.; 2% X 1890

Fig. 8. Spindle fibres pushing chromosomes

2-1/4 expos. ; 2% X 1890

Fig. 9. Excessive production of spindle fibres - Extra-nuclear chromatin

2-1/4 expos.; 2 % X 1890

Fig. 10. Diagonal spindles

2 -1/4 expos.; 2% X 995

Fig. 11. Disintegrated spindle - Extra-Nuclear chromatin

2-1/4 expos.; 2% X 1890

Fig. 12. Similar to above. No cell plate

2-1/4 expos.; 2% X 1890

Fig. 13. Crescentic spindle

1/2 expos.; 2% X 1890

Fig. 14. Disintegrating spindle - No cell plate

2-1/2 expos.; 2% X 995

Fig. 15. Distortion of cell plate

1/2 expos. ; 2% X 1890

Fig. 16. Bath nuclei of a binucleated cell in stage of division

2 expos.; 43 reg. ; 2 % X 1890

Fig. 17. Binucleated cell

1 expos.; 24 reg. 2% X 1890

Fig. 18. Pseudoamitosis

2 expos.; 24 reg.; 2% X 1890

Fig. 19. Pseudoamitosis

2 expos.; 24 reg.; 2% X 1890

Fig. 20. Unequal division, due to distortion of spindle

2-1/2 expos.; 2-1/2% X 1890

Fig. 21. Formation of resting stage

2 expos.; 2% X 1890

Fig. 22. Same as above, also formation of extra-nuclear chromatin masses

2-1/4 expos. 2% X 1890

Fig. 23. Formation of resting stage, with chromatin bridge connecting masses

2-1/4 expos.; 2% X 1890

Fig. 24. Origin of bi-nucleated cell. Distorted spindle

2-1/2 expos.; 2 % X 1890

Fig. 25. Two polar masses of chromatin. Origin of resting stage, and origin of extra-nuclear chromatin masses

2-1/4 expos.; 2% X 1890

Fig. 26. "a", "b", "c", three views of such a mass as is shown in Fig. 25, from three planes of focus

2-1/4 expos.; 2% X 1890

Fig. 27. Nucleus from near tip of root to show hof

2-1/2 expos.; 2-1/2% X 2500

Fig. 28. Nucleus from same section farther up, to show large nucleolus without hof

2-1/2 expos.; 2 -1/2 % X 2500

Fig. 29. Nucleus whose nucleolus is scarcely distinguishable from chromatin net. From same section as Fig. 27

2-1/2 expos.; 2-1/2% X 2500

Fig. 30. Nucleus with smaller nucleolus than in Fig. 28. From same section as Fig. 28

2-1/2 expos.; 2-1/2 % X 2500

Fig. 31. Nucleus with no nucleolus. From same section as Fig2 8

2-1/2 expos.; 2-1/2 % X 2500

Fig. 32. Stage between Figs. 28 and 30.

2-1/2 expos.; 2-1/2 % X 2500

Figs. 33 & 34.

Surface views of nuclei to show their irregular shape

2 -1/2 expos.; 2-1/2 % X 2500

Fig. 35. Group of cells to show haphazard distribution of chromosomes in some cells

1-1/2 expos.; 2% X 995

Figs. 36 & 37.

Disintegrating spindles. No cell plate. Origin of bi-nucleated cells

2-1/4 expos.; 2% X 995

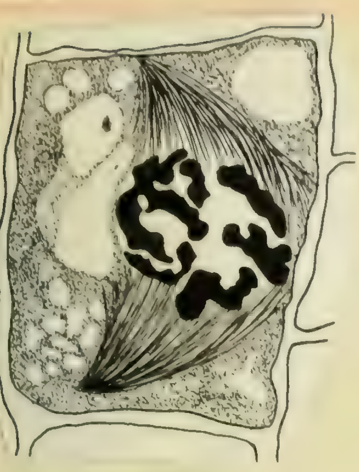
Figs. 38, 39 & 40.

Spindles much bent. In Fig. 40 the whole mass of chromatin is about to re-fuse into one nucleus

2-1/4 expos.; 2% X 995

Fig. 41. Similar to Fig. 40. Re-fusion of chromatin. Spindle not in intact as in Fig. 40

2-1/4 expos.; 2 % X 995



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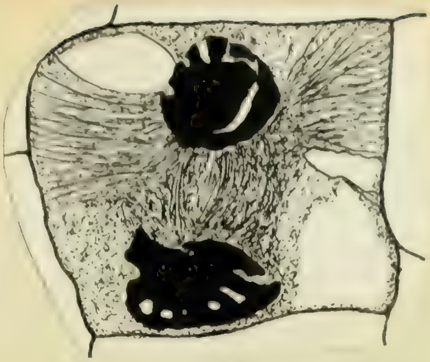

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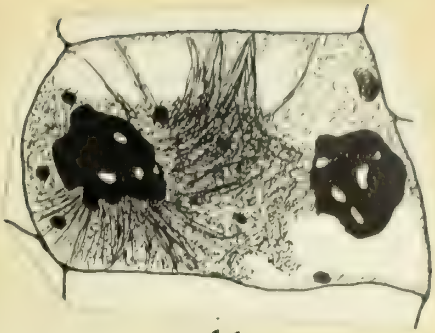

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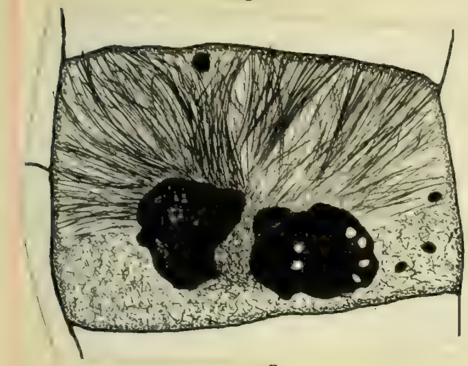

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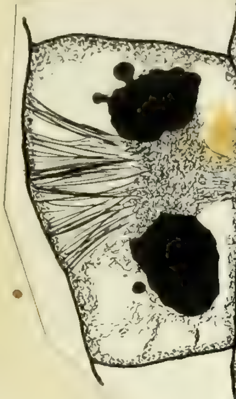

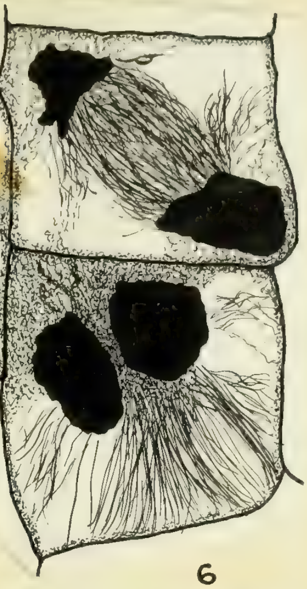

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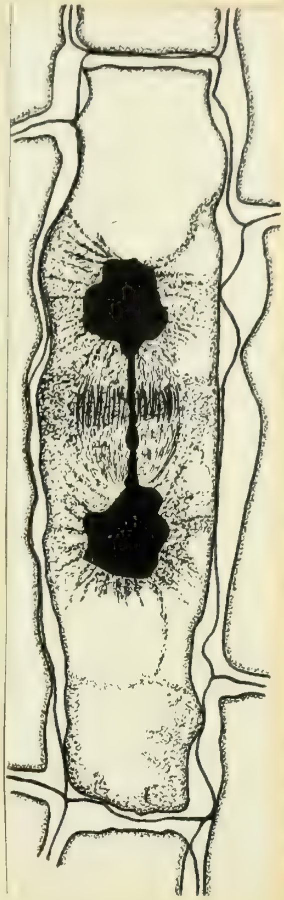

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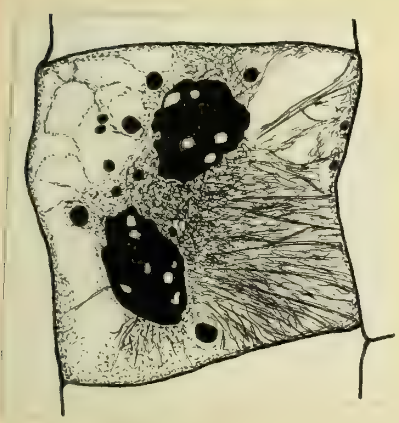

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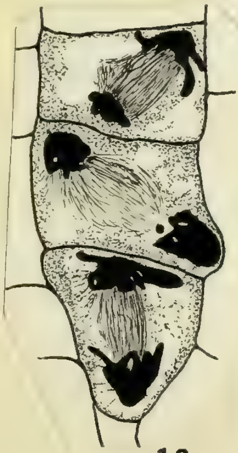

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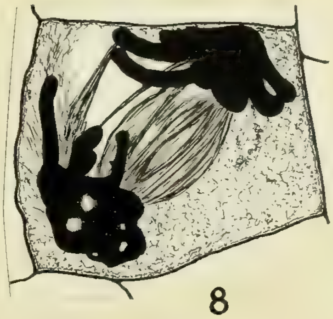

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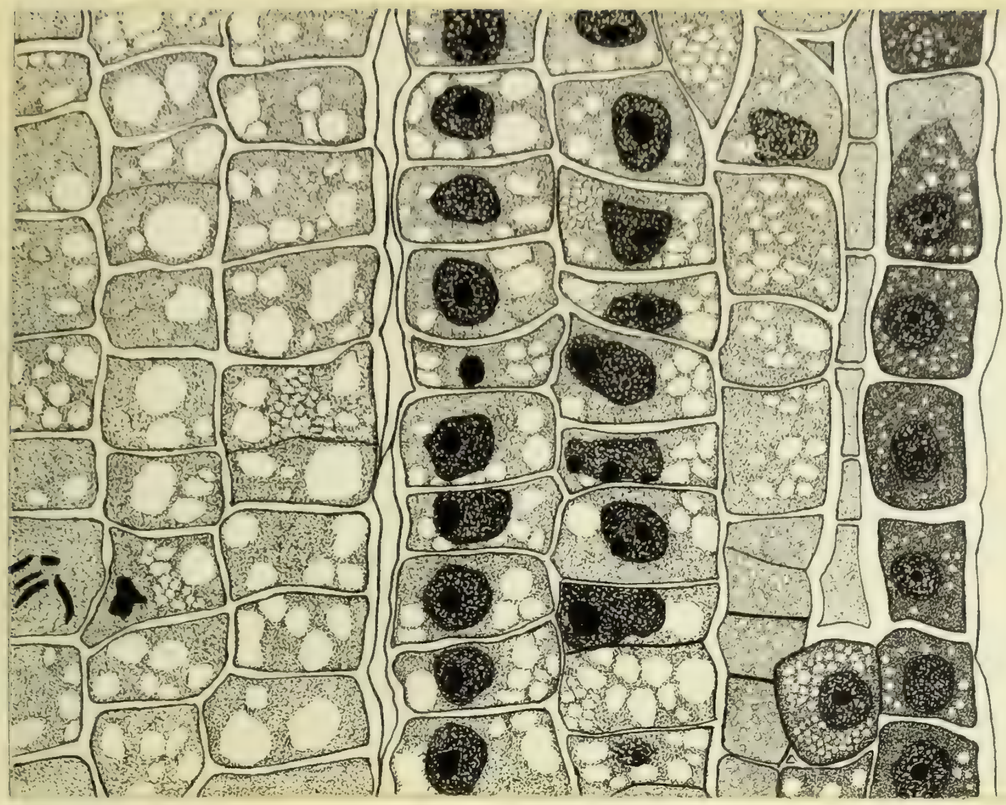

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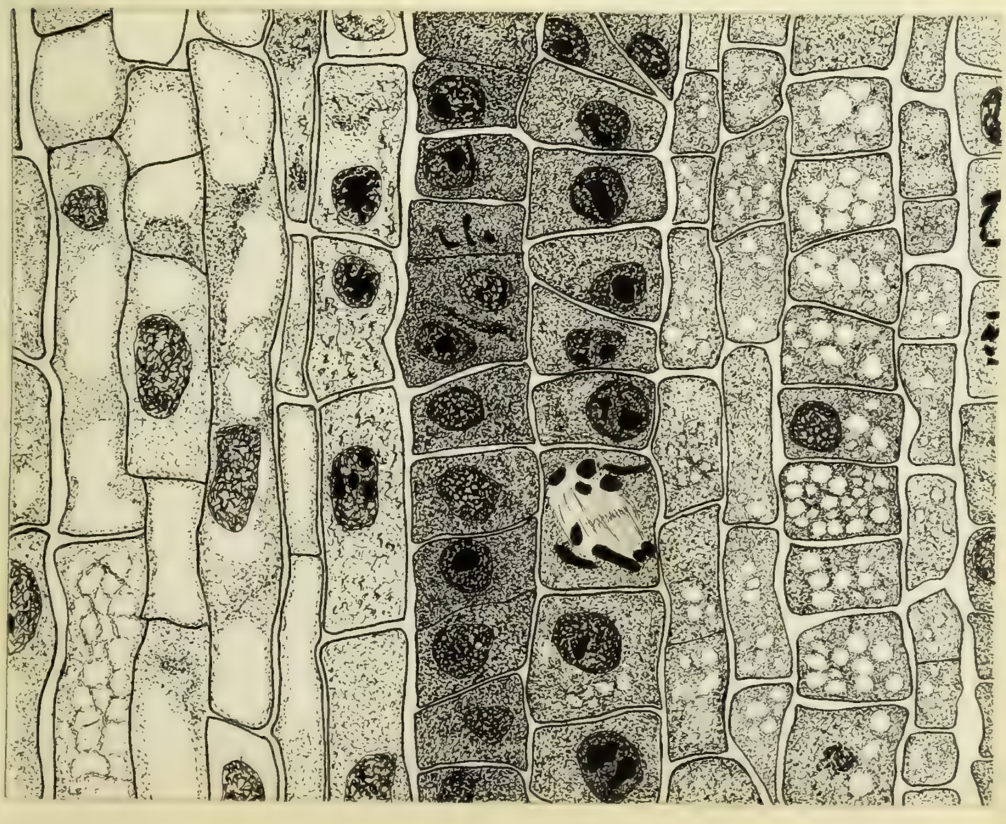

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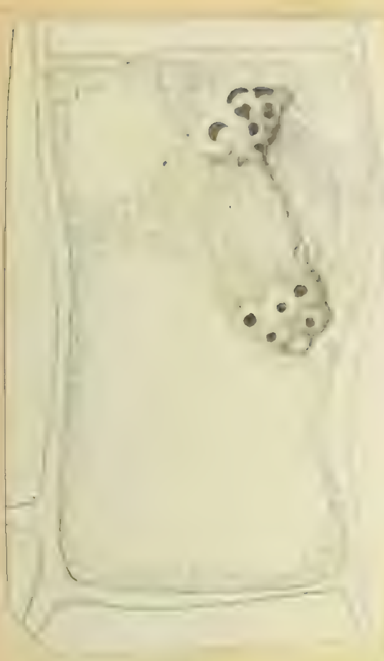

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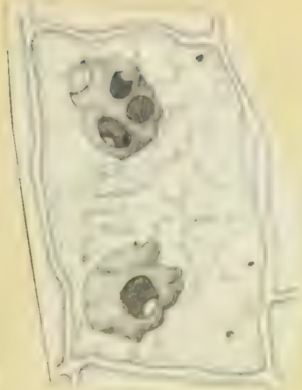

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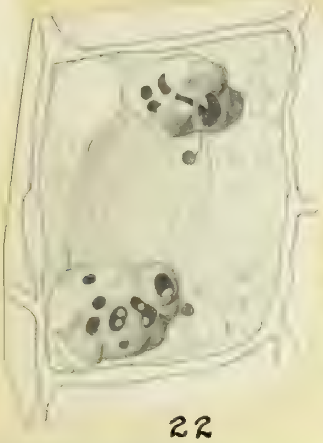

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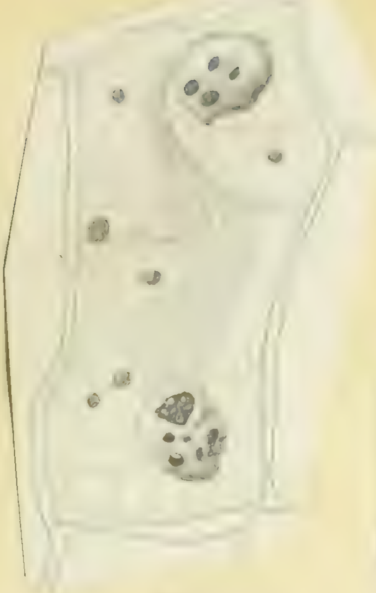

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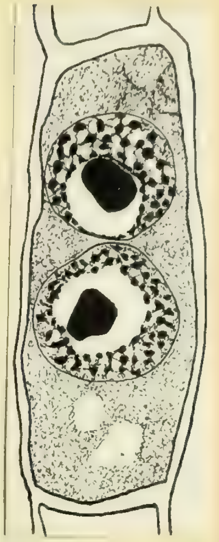

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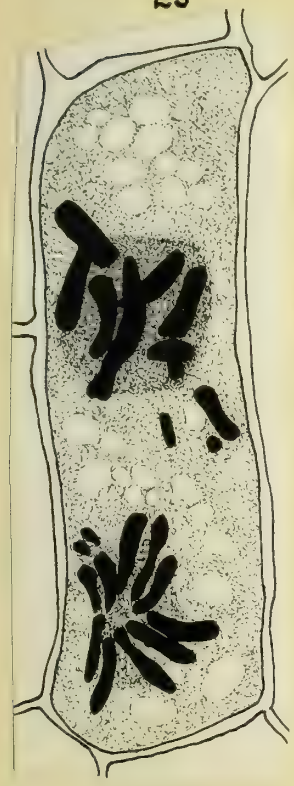

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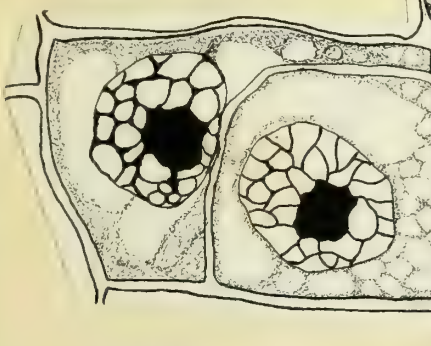

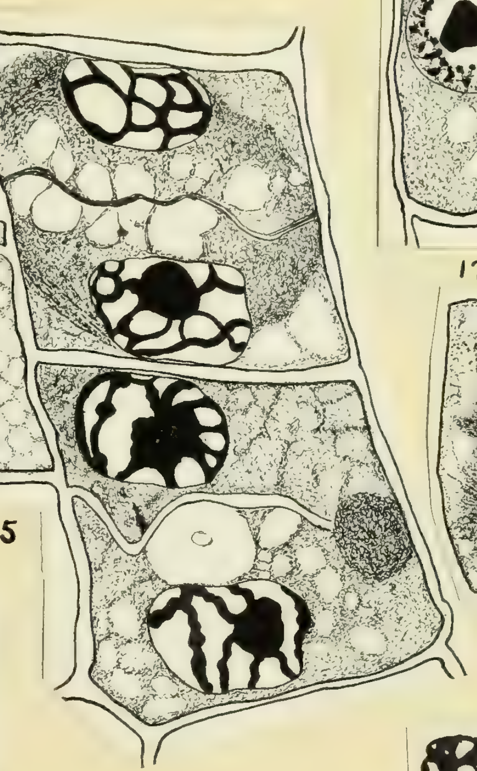

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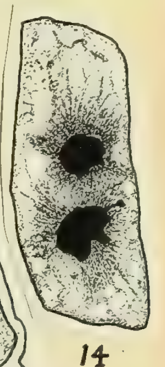

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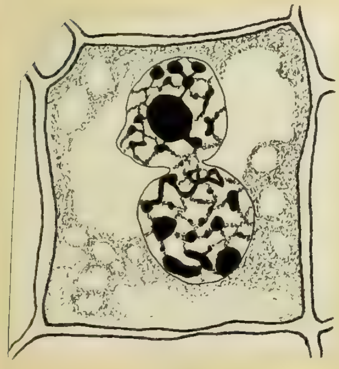

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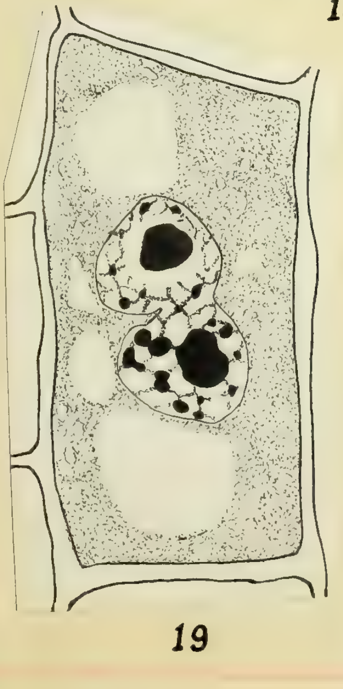

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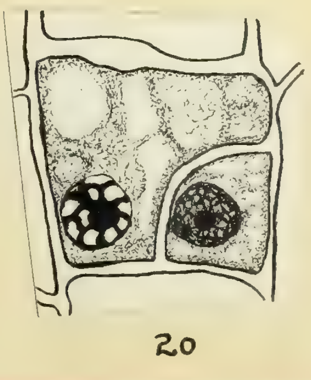

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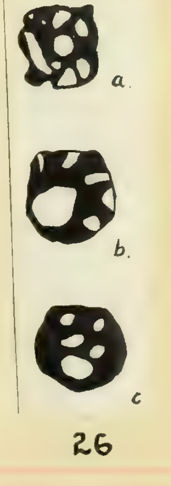

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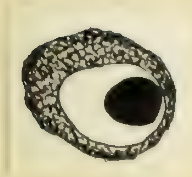

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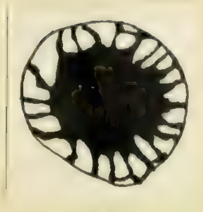

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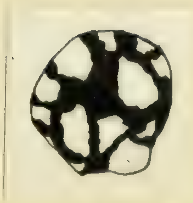

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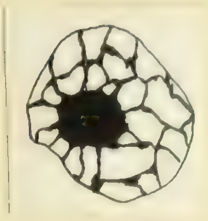

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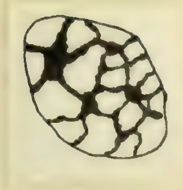

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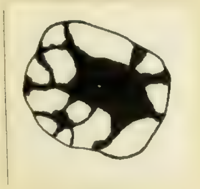

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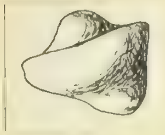

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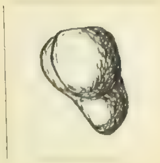

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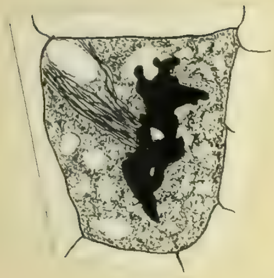

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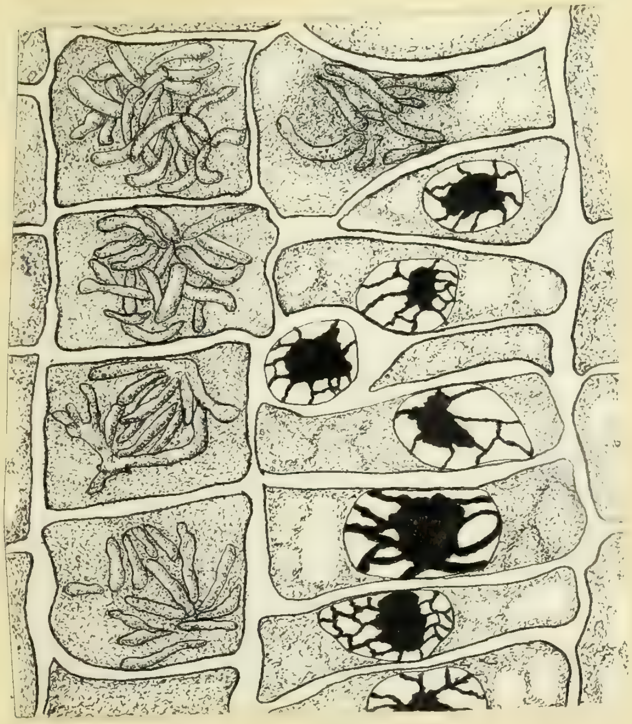

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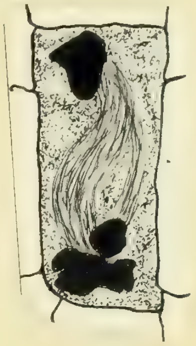

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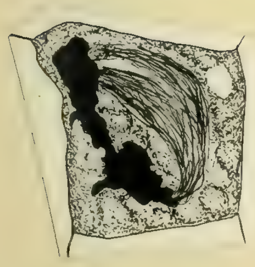

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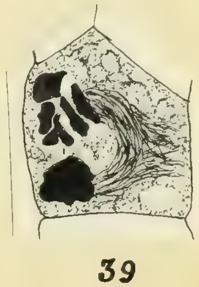

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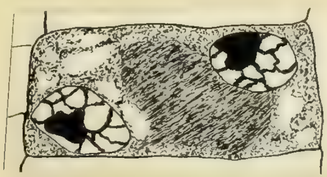

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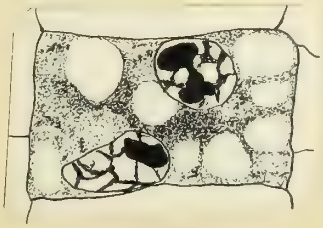

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